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Chem-Bio News – S&T Supplement

1. SUBSTRATE BINDING MODE AND ITS IMPLICATION ON DRUG DESIGN FOR

BOTULINUM NEUROTOXIN A: *"Here, we report the crystal structures of the catalytic domain of BoNT/A with its uncleavable SNAP-25 peptide 197QRATKM202 and its variant 197RRATKM202 to 1.5 Å and 1.6 Å, respectively. This is the first time the structure of an uncleavable substrate bound to an active botulinum neurotoxin is reported and it has helped in unequivocally defining S1 to S5' sites."*

2. THE DIPHTHAMIDE MODIFICATION ON ELONGATION FACTOR-2 RENDERS

MAMMALIAN CELLS RESISTANT TO RICIN: *"These data show that the presence of diphthamide in eEF-2 provides protection against ricin and suggest the hypothesis that diphthamide may have evolved to provide protection against RIPs [ribosome-inactivating protein]."*

3. DIFFERENTIAL ANTIGEN REQUIREMENTS FOR PROTECTION AGAINST SYSTEMIC

AND INTRANASAL VACCINIA VIRUS CHALLENGES IN MICE: *"These studies also suggest that rAd vectors warrant further assessment as candidate subunit smallpox vaccines."*

4. POLY(D,L-LACTIDE-CO-GLYCOLIDE) NANOPARTICLE AGGLOMERATES AS

CARRIERS IN DRY POWDER AEROSOL FORMULATION OF PROTEINS: *"Controlled flocculation of nanoparticles may provide a useful alternative to spray drying when formulating dry powders for pulmonary or nasal administration of protein therapeutics or antigens."*

5. MONOVALENT VIRUS-LIKE PARTICLE VACCINE PROTECTS GUINEA PIGS AND

NONHUMAN PRIMATES AGAINST INFECTION WITH MULTIPLE MARBURG VIRUSES: *"Musoke mVLPs [Marburg virus-like particle] are effective at inducing broad heterologous immunity and protection against multiple MARV isolates."*

6. DIFFERENT MECHANISMS OF CELL ENTRY BY HUMAN-PATHOGENIC OLD WORLD

AND NEW WORLD ARENAVIRUSES: *"Our data indicate that LASV enters cells via a pathway distinct from the one used by human-pathogenic New World arenaviruses."*

7. DETECTION OF RICIN IN COMPLEX SAMPLES BY IMMUNOCAPTURE AND MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS

SPECTROMETRY: *"The present assay provides a new tool with a total analytical time of approximately 5 h, which is particularly relevant in the context of a bioterrorist incident."*

CB Daily Report

Chem-Bio News

SUBSTRATE BINDING MODE AND ITS IMPLICATION ON DRUG DESIGN FOR BOTULINUM NEUROTOXIN A

By Desigan Kumaran, Richa Rawat, S. Ashraf Ahmed, Subramanyam Swaminathan
PLoS Pathogens
October 3, 2008

"The seven antigenically distinct serotypes of *Clostridium botulinum* neurotoxins, the causative agents of botulism, block the neurotransmitter release by specifically cleaving one of the three SNARE proteins and induce flaccid paralysis. The Centers for Disease Control and Prevention (CDC) has declared them as Category A biowarfare agents. The most potent among them, botulinum neurotoxin type A (BoNT/A), cleaves its substrate synaptosome-associated protein of 25 kDa (SNAP-25). An efficient drug for botulism can be developed only with the knowledge of interactions between the substrate and enzyme at the active site. Here, we report the crystal structures of the catalytic domain of BoNT/A with its uncleavable SNAP-25 peptide 197QRATKM202 and its variant 197RRATKM202 to 1.5 Å and 1.6 Å, respectively. This is the first time the structure of an uncleavable substrate bound to an active botulinum neurotoxin is reported and it has helped in unequivocally defining S1 to S5' sites. These substrate peptides make interactions with the enzyme predominantly by the residues from 160, 200, 250 and 370 loops. Most notably, the amino nitrogen and carbonyl oxygen of P1 residue (Gln197) chelate the zinc ion and replace the nucleophilic water. The P1'-Arg198, occupies the S1' site formed by Arg363, Thr220, Asp370, Thr215, Ile161, Phe163 and Phe194. The S2' subsite is formed by Arg363, Asn368 and Asp370, while S3' subsite is formed by Tyr251, Leu256, Val258, Tyr366, Phe369 and Asn388. P4'-Lys201 makes hydrogen bond with Gln162. P5'-Met202 binds in the hydrophobic pocket formed by the residues from the 250 and 200 loop. Knowledge of interactions between the enzyme and substrate peptide from these complex structures should form the basis for design of potent inhibitors for this neurotoxin."

The full article can be found at: <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000165;jsessionid=5D3E28D1E98806FB1F959C50109EB122>

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THE DIPHTHAMIDE MODIFICATION ON ELONGATION FACTOR-2 RENDERS

MAMMALIAN CELLS RESISTANT TO RICIN

Drug Week

October 10, 2008

"Chinese hamster ovary (CHO) cells mutated in the biosynthetic genes lack diphthamide and are resistant to bacterial toxins such as diphtheria toxin. We found that diphthamide-deficient cultured cells were threefold more sensitive than their parental cells towards ricin, a ribosome-inactivating protein (RIP). RIPs bind to ribosomes at the same site as eEF-2 and cleave the large ribosomal RNA, inhibiting translation and causing cell death. We hypothesized that one role of diphthamide may be to protect ribosomes, and therefore all eukaryotic life forms, from RIPs, which are widely distributed in nature. A protective role of diphthamide against ricin was further demonstrated by complementation where dph mutant CHO cells transfected with the corresponding DPH gene acquired increased resistance to ricin in comparison with the control-transfected cells, and resembled the parental CHO cells in their response to the toxin."

"These data show that the presence of diphthamide in eEF-2 provides protection against ricin and suggest the hypothesis that diphthamide may have evolved to provide protection against RIPs."

The full article can be found at: (P.K. Gupta, et. al., "The diphthamide modification on elongation factor-2 renders mammalian cells resistant to ricin". Cellular Microbiology, 2008; 10(8):1687-1694). Link not available.

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DIFFERENTIAL ANTIGEN REQUIREMENTS FOR PROTECTION AGAINST SYSTEMIC AND INTRANASAL VACCINIA VIRUS CHALLENGES IN MICE

Medicine & Law Weekly

October 10, 2008

"Here we explore the protective efficacy of replication-incompetent, recombinant adenovirus serotype 35 (rAd35) vectors expressing the vaccinia virus intracellular mature virion (IMV) antigens A27L and L1R and extracellular enveloped virion (EEV) antigens A33R and B5R in a murine vaccinia virus challenge model. A single immunization with the rAd35-L1R vector effectively protected mice against a lethal systemic vaccinia virus challenge. The rAd35-L1R vector also proved more efficacious than the combination of four rAd35 vectors expressing A27L, L1R, A33R, and B5R. Moreover, serum containing L1R-specific neutralizing antibodies afforded postexposure prophylaxis after systemic vaccinia virus infection. In contrast, the combination of rAd35-L1R and rAd35-B5R vectors was required to protect mice against a lethal intranasal vaccinia virus challenge, suggesting that both IMV- and EEV-specific immune responses are important following intranasal infection. Taken together, these data demonstrate that different protective antigens are required based on the route of vaccinia virus challenge."

"These studies also suggest that rAd vectors warrant further assessment as candidate subunit smallpox vaccines."

The full article can be found at: (D.R. Kaufman, et. al., "Differential antigen requirements for protection against systemic and intranasal vaccinia virus challenges in mice". *Journal of Virology*, 2008; 82(14):6829-6837). Link not available.

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POLY(D,L-LACTIDE-CO-GLYCOLIDE) NANOPARTICLE AGGLOMERATES AS CARRIERS IN DRY POWDER AEROSOL FORMULATION OF PROTEINS

Science Letter

October 7, 2008

"A dry powder aerosol drug delivery system was designed with both nano- and microstructure to maximize the protein loading via Surface adsorption and to facilitate delivery to the deep lung, respectively. Ovalbumin was employed as a model protein to adsorb to and controllably flocculate DOTAP-coated PLG nanoparticles into "nanoclusters" possessing low density microstructure."

"The mechanism of nanoparticle flocculation was probed by evaluating the effects of ionic strength, shear force, and protein concentration on the geometric and aerodynamic diameters of the nanoclusters as well as the protein adsorption efficiency. Salt ions were found to compete with ovalbumin adsorption to nanoparticles and facilitate flocculation; therefore, formulation of nanoclusters for inhaled drug delivery may require the lowest possible ionic strength to maximize protein adsorption. Additional factors, such as shear force and total protein-particle concentration can be altered to optimize nanocluster size, suggesting the possibility of regional lung delivery. Immediate release of ovalbumin was observed, and native protein structure upon release was confirmed by circular dichroism and fluorescence spectroscopy studies."

"Controlled flocculation of nanoparticles may provide a useful alternative to spray drying when formulating dry powders for pulmonary or nasal administration of protein therapeutics or antigens."

The full article can be found at: (L.J. Peek, et. al., "Poly(D,L-lactide-co-glycolide) nanoparticle agglomerates as carriers in dry powder aerosol formulation of proteins". *Langmuir*, 2008; 24(17):9775-9783). Link not available.

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MONOVALENT VIRUS-LIKE PARTICLE VACCINE PROTECTS GUINEA PIGS AND NONHUMAN PRIMATES AGAINST INFECTION WITH MULTIPLE MARBURG VIRUSES

Blood Weekly

October 16, 2008

"Virus-like particle (VLP)-based vaccines have the advantage of being morphologically and

antigenically similar to the live virus from which they are derived, Expression of the glycoprotein and VP40 matrix protein from Lake Victoria marburgvirus (MARV) results in spontaneous production of VLPs in mammalian cells. Guinea pigs vaccinated with Marburg virus VLPs (mVLPs) or inactivated MARV (iMARV) develop homologous humoral and T-cell responses and are completely protected from a lethal homologous MARV challenge."

"To determine whether mVLPs based on the Musoke (aka Lake Victoria) isolate of MARV could broadly protect against diverse isolates of MARV guinea pigs were vaccinated with mVLPs or iMARV-Musoke and challenged with MARV-Musoke, -Ravn or Ci67. Prior to challenge, the mVLP- and iMARV-vaccinated guinea pigs had high levels of homologous MARV-Musoke and heterologous MARV-Ravn and -Ci67 antibodies. The Musoke-based mVLPs and iMARV vaccines provided complete protection in guinea pigs against viremia, viral replication and pathological changes in tissues, and lethal disease following challenge with MARV-Musoke, -Ravn or -Ci67. Guinea pigs vaccinated with RIBI adjuvant alone and infected with guinea pig-adapted MARV-Musoke, -Ravn or -Ci67 had histopathologic findings similar to those seen in the nonhuman primate model for MARV infection. Based on the strong protection observed in guinea pigs, we next vaccinated cynomolgus macaques with Musoke-based mVLPs and showed the VLP-vaccinated monkeys were broadly protected against three isolates of MARV (Musoke, Ravn and Ci67)."

"Musoke mVLPs are effective at inducing broad heterologous immunity and protection against multiple MARV isolates."

The full article can be found at: (D.L. Swenson, et. al., "Monovalent virus-like particle vaccine protects guinea pigs and nonhuman primates against infection with multiple Marburg viruses". Expert Review of Vaccines, 2008; 7(4):417-429). Link not available.

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DIFFERENT MECHANISMS OF CELL ENTRY BY HUMAN-PATHOGENIC OLD WORLD AND NEW WORLD ARENAVIRUSES

Hematology Week
October 13, 2008

"The Old World arenavirus Lassa virus (LASV) is the causative agent of severe viral hemorrhagic fever (VHF) in humans and is the most prevalent human pathogen among arenaviruses. The present study investigated the largely unknown mechanisms of cell entry of LASV, a process known to be mediated solely by the virus envelope glycoprotein (GP)."

"To circumvent biosafety restrictions associated with the use of live LASV, we used reverse genetics to generate a recombinant variant of the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) expressing the LASV GP (rLCMV-LASVGP). The rescued rLCMV-LASVGP grew to titers comparable to that of LCMV and showed the receptor binding characteristics of LASV. We used rLCMV-LASVGP to characterize the cellular mechanisms of LASV entry in the context of a productive arenavirus infection. The kinetics of pH-dependent membrane fusion of rLCMV-LASVGP resembled those of the human-pathogenic New World arenavirus Junin virus (JUNV) and other enveloped viruses that use clathrin-mediated

endocytosis for entry. However, rLCMV-LASVGP entered cells predominantly via a clathrin-, caveolin-, and dynamin-independent endocytotic pathway similar to the one recently described for LCMV. Productive infection of rLCMV-LASVGP was only mildly affected by a dominant negative mutant of Rab5 and was independent of Rab7, suggesting an unusual mechanism of delivery to endosomes. In addition, rLCMV-LASVGP infection was independent of actin but required intact microtubules."

"Our data indicate that LASV enters cells via a pathway distinct from the one used by human-pathogenic New World arenaviruses."

The full article can be found at: (J.M. Rojek, et. al., "Different mechanisms of cell entry by human-pathogenic old world and New World arenaviruses". Journal of Virology, 2008;82 (15):7677-7687). Link not available.

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DETECTION OF RICIN IN COMPLEX SAMPLES BY IMMUNOCAPTURE AND MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

Drug Week

October 17, 2008

"Here, we report a method combining immunocapture and analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the accurate detection of different species of *R. communis*. Liquid environmental samples were applied to magnetic particles coated with a monoclonal antibody directed against the B-chain of the toxin. After acidic elution, tryptic peptides of the A- and B-chains were obtained by accelerated digestion with trypsin in the presence of acetonitrile. Of the 20 peptides observed by MALDI-TOF MS, three were chosen for detection (m/z 1013.6, m/z 1310.6 and m/z 1728.9, which correspond to peptides 161-LEQLAGNLR-169, 150-YTFAFGGNYDR-160, and 233-SAPDPSVITLENSWGR-248, respectively). Their selection was based on several parameters such as detection sensitivity, specificity toward ricin forms and absence of isotopic overlap with unrelated peptides. To increase assay reproducibility, stable isotope-labeled peptides were incorporated during the sample preparation phase. The final assay has a limit of detection estimated at approximately 50 ng/mL (approximately 0.8 nM) of ricin in buffer. No interference was observed when the assay was applied to ricin-spiked milk samples. In addition, several varieties of *R. communis* or from different geographical origins were also shown to be detectable."

"The present assay provides a new tool with a total analytical time of approximately 5 h, which is particularly relevant in the context of a bioterrorist incident."

The full article can be found at: (E. Duriez, et. al., "Detection of ricin in complex samples by immunocapture and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry". Journal of Proteome Research, 2008;7(9):4154-63). Link not available.

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